OMEGA bio-tek

Innovations in nucleic acid isolation

Product Manual

E.Z.N.A.® Poly-Gel DNA Extraction Kit

D2561-00	5 preps
D2561-01	20 preps
D2561-02	50 preps

May 2013

For research use only.Not intended for diagnostic testing.

E.Z.N.A.® Poly-Gel DNA Extraction Kit

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Introduction and Overview

While advances in oligonucleotide synthesis have allowed rapid, facile and efficient production of DNA fragments, purification can be time-consuming and occasionally ineffective. In some applications, there is no need for purified product. However, when purification is necessary, the two traditional techniques used for the purification of synthetic oligonucleotides or double stranded DNA fragments ≤ 100 bp are polyacrylamide gel electrophoresis (PAGE) and high performance liquid chromatography (HPLC). While HPLC remains a potent analytical tool, PAGE and its distant cousin capillary electrophoresis (CE) are probably most often used for the analysis of synthetic oligonucleotides. With UV shadowing (or ethidium bromide staining), PAGE allows the rapid purification of small quantities of synthetic DNA and is especially useful for parallel purification of several samples.

The E.Z.N.A.® Poly-Gel DNA Extraction Kit combines the simplicity of PAGE with the power of HiBind® technology to allow rapid and consistent recovery of ssDNA or dsDNA from acrylamide gels. Following PAGE, oligonucleotides are first visualized by UV shadowing or ethidium bromide staining and then eluted in 1-4 hours using EDTA-containing buffer to eliminate degradation of DNA by contaminating DNase. A specially formulated buffer is then added to the eluate which is passed through a HiBind® DNA Mini Column. The bound DNA is washed to remove salts, buffers, and any protein contaminants, and then eluted with sterile deionized water or low salt buffer. Organic extractions and alcohol precipitations are eliminated reducing sample handling time and increasing yields. Furthermore, the spin column format allows parallel processing of multiple samples. The purified DNA is suitable for PCR, ligation reactions, hybridization techniques, or other procedures.

New in this Edition: This manual has been edited for content and redesigned to enhance user readability.

Kit Contents

Product	D2561-00	D2561-01	D2561-02
Purifications	5 preps	20 preps	50 preps
HiBind® DNA Mini Columns	5	20	50
Poly-Gel Filter Units	5	20	50
2 mL Collection Tubes	5	20	50
Poly-Gel Elution Buffer	5 mL	15 mL	25 mL
HBC Buffer	8 mL	25 mL	50 mL
DNA Wash Buffer	1.5 mL	9 mL	15 mL
User Manual	✓	✓	✓

Storage and Stability

All of the E.Z.N.A.® Poly-Gel DNA Extraction Kit components are guaranteed for at least 12 months from the date of purchase when stored at room temperature. During shipment or storage in cool ambient conditions, precipitates may form in some of the buffers. Dissolve such deposits by warming the solution at 37°C and gently shaking.

Preparing Reagents

- Dilute DNA Wash Buffer with 100% ethanol as follows and store at room temperature.

Kit	100% Ethanol to be Added
D2561-00	6 mL
D2561-01	36 mL
D2561-02	60 mL

- Dilute HBC Buffer with isopropanol as follows and store at room temperature.

Kit	Isopropanol to be Added
D2561-00	3.2 mL
D2561-01	10 mL
D2561-02	20 mL

Acrylamide gels are useful for separation of small DNA fragments, typically oligonucleotides <100 base pairs. These gels are usually of a low acrylamide concentration (≤6%) and contain the non-ionic denaturing agent urea (7 M). For gel composition and buffers for your particular requirements, please refer to the Acrylamide Gel section on Page 8.

Visualization of DNA

Fluorescence Shadowing

DNA fragments resolved on polyacrylamide gels can be visualized by the method of UV shadowing. This method is straightforward and may give higher yields compared to staining methods (see DNA Staining below). In this technique, the gel is placed on top of a fluorescent material, usually a fluorescent TLC silica plate. The gel is then illuminated by a UV light source. DNA bands in the gel will block transmittance of the UV light to the substrate. This will result in a dark area (i.e. non-fluorescent) area on the substrate.

1. The gel must be removed from glass plates (glass blocks UV light and will prevent visualization by either UV shadowing or staining) and wrapped in plastic wrap to aid in handling and marking. Remove the top glass plate, and lay a sheet of plastic wrap over the gel, then flip the gel & glass plate over and carefully peel the gel away from the bottom glass plate. Wrap the gel entirely in the plastic wrap.

   Tip: Use only a single layer of plastic wrap and try to prevent air bubbles from forming between the gel and plastic wrap. These bubbles can scatter the UV light and make visualization difficult.

2. Place the gel on top of the dull white side of a fluor-coated TLC plate (Ambion, 10110 or Merck 60F254) and remove the plastic wrap on top of the gel. Hold a hand-held short-wavelength (254 nm) UV light source over the gel. Long wavelength UV light will not work. The TLC plate beneath the gel should glow bright purple except wherever nucleic acids are present. A DNA band will appear as a dark shadow. The limit of sensitivity is about 0.3 µg in a single band.

   Tip: UV shadowing works for either DNA or RNA, labeled or unlabeled, so this technique has many other applications; e.g. for visualizing restriction enzyme digests.

DNA Staining

As an alternative to UV shadowing the acrylamide gel may be stained with acridine orange or ethidium bromide (EtBr) and held over a UV trans-illuminator to visualize the location of DNA bands within the gel.

If the DNA is to used as a probe, it is important that the stain be completely removed before hybridization as it will compromise hybridization efficiency. We recommend staining with acridine orange as opposed to EtBr since acridine orange will be removed from the probe by subsequent purification with HiBind® DNA Mini Columns. EtBr can be used, but requires multiple butanol extractions to remove it before applying sample to the HiBind® DNA Mini Column.

1. Remove gel from glass plates as described for UV shadowing.

2. Remove gel from the plastic wrap and place in a 2.0 µg/mL acridine orange solution for 15 minutes. Destain the gel in distilled water for 10 minutes. Then rewrap the gel in plastic wrap for easier handling and place the gel on a UV trans-illuminator to visualize DNA.

3. Carefully cut out (using a nuclease-free scalpel or razor blade) the smallest gel-fragment possible which contains the DNA band (corresponds to bright band in the gel). The smaller the size of this gel fragment, the better the elution efficiency (i.e. more probe will be recovered more quickly). If you are concerned that not all the probe was cut out, visualize the gel again with UV light to verify that the probe band is gone.

Isotopically Labeled DNA

If the DNA band of interest has been labeled with ^{32}P, ^{33}P, or ^{35}S as the probe, it can be readily visualized by autoradiography.

1. Following PAGE, separate the glass plates, leaving the gel adherent to the larger glass plate. Wrap a piece of plastic wrap over the gel. If the glass and gel will not fit into the film cartridge, then both glass plates should be carefully removed and the gel wrapped entirely in plastic wrap (for easier handling).The gel is ready to expose to film.

2. Place the gel (sandwiched between the glass and plastic wrap) against the film so that the film is closest to the gel. The film can simply be aligned with one corner of the glass plate, the corners and sides of the glass plate marked directly on the film with a permanent marker, or alternatively, radioactive ink can be used for orientation. One corner of the film (e.g. bottom right corner) is usually snipped or folded up so that the glass and gel can be aligned with the film after developing.

3. Expose the gel to autoradiographic film, about 30 seconds for a high specific activity ^{32}P-labeled probe and 10 minutes for a low specific activity ^{33}P-labeled probe or high specific activity ^{35}S-labeled probe. The aim is to get an exposure of a light gray band so that a thin gel fragment can be excised from the gel. Realign the glass plate and gel with the developed film (using the guide marks made earlier) and carefully excise the band using a nuclease-free scalpel or razor blade. The smaller the size of this gel fragment, the better the elution efficiency (i.e. more probe will be recovered more quickly). The gel can be re-exposed to insure that the gel and film were properly aligned and that the probe was excised.

   Tip: If possible, run markers or a known size standard so that the appropriate band is selected. If no markers have been run, the bromophenol (dark blue) and xylene cyanol (light blue) dyes can serve as size references. See Acrylamide Gel section on Page 8 for dye migration under different conditions.

Acrylamide Gels

1. Choose the proper gel concentration from the table below.

Gel Concentration	Separation Range	bp corresponding to bromophenol blue	bp corresponding to xylene cyanol
Polyacrylamide (30:1) - ds linear DNA			
3.5%	25-500	100	450
5.0%	15-300	60	270
8.0%	5-200	25	150
Urea-polyacrylamide (19:1) - ss linear DNA			
5.0%		30	125
6.0%		25	110
8.0%		20	75
20.0%		7	27

2. Prepare running buffer as follows: 100 mM Tris-Borate pH 8.3, 2 mM EDTA.

3. Prepare the acrylamide-bisacrylamide (29:1) gel with 7 M urea, 100 mM Tris-borate, and 2 mM EDTA.

Final Acrylamide Concentration	8%	12%	20%
40% acrylamide-bisacrylamide (29:1)	12 mL	18 mL	30 mL
Urea	30 g	30 g	30 g
Distilled water	18.5 mL	12.5 mL	0.5 mL
1.0 M Tris-borate (pH 8.3), 20 mM EDTA	6 mL	6 mL	6 mL
10% ammonium persulfate	420 µL	420 µL	420 µL
TEMED	20 µL	20 µL	20 µL

4. Mix all the ingredients, except the ammonium persulfate and TEMED in a round bottle and evacuate to degas. Add ammonium persulfate and TEMED just prior casting the gel. 60 mL is enough for a 400 x 200 x 0.5 mm gel.

E.Z.N.A.® Poly-Gel DNA Extraction Kit Protocol

Please take a few minutes to read and familiarize yourself with this procedure. Make sure all necessary reagents and equipment are ready before starting.

Materials and Equipment to be Supplied by User:

- Microcentrifuge capable of 10,000 x *g*
- Water bath, heat block, or incubator capable of 65°C
- Nuclease-free 1.5 mL microcentrifuge tubes
- Nuclease-free 1 mL pipette tips
- Nuclease-free microscope slides
- Nuclease-free razor
- 100% Ethanol
- Sterile deionized H_2O
- Optional: Autoradiographic film for isotopically labeled DNA

Before Starting:

- Prepare DNA Wash Buffer according to the Preparing Reagents section on Page 4
- Set a water bath, heat block, or incubator to 65°C

1. Transfer the gel fragment onto a nuclease-free microscope slide. With a second glass slide or nuclease-free razor, mash and pulp the gel completely.

2. Carefully transfer gel pulp to a nuclease-free microcentrifuge tube.

3. Add 250 µL Poly-Gel Elution Buffer.

   Note: This volume is usually enough to submerge a slice 2 mm x 10 mm x 0.8 mm. For a larger fragment adjust volume of Poly-Gel Elution Buffer used until the gel is covered. Any buffer or diH_2O can be used; however, we recommend the Elution Buffer supplied in order to prevent DNA degradation by exogenous nucleases.

4. Incubate at 65°C for 1-4 hours.

   Note: The elution time is dependent on the size of the gel fragment, DNA size, and incubation temperature. We find that about 70% of a 100 bp DNA fragment elutes in approximately 4 hours at 65°C. Larger fragments will take longer to elute.

5. Transfer sample to a Poly-Gel Filter unit mounted in a sterile 1.5 ml microcentrifuge tube. Use a 1 mL pipette tip with the end cut to do this.

6. Centrifuge at 10,000 x *g* 10 minutes at room temperature.

   Note: If isolating labeled DNA probes for use in hybridization assays, no further purification is necessary. An aliquot of the eluted probe can be used directly in the hybridization reaction. An optional phenol: chloroform extraction may be performed. However do not extract with phenol if the DNA probe is labeled with digoxygenin as DNA will separate into the organic phase. Also a standard ethanol precipitation with carrier (glycogen, tRNA, or linear acrylamide) may be performed for further clean up.

7. Use eluate directly if isolating labeled DNA probes for use in hybridization assays. For downstream applications that require enzymatic manipulation of DNA, such as with PCR, continue to Step 8 below.

DNA Clean Up with HiBind® DNA Mini Columns

8. Add 4.5-5 volumes HBC Buffer. Vortex briefly to mix.

   Note: HBC Buffer must be diluted with isopropanol before use. Please see Page 4 for instructions.

   Note: For fragments smaller than 100 bp, use at least 6 volumes HBC Buffer. Adding 5-10 µg yeast tRNA as a carrier will also increase the yield of DNA recovered. The volume of carrier tRNA should be no more than 1/10 the volume of the HBC Buffer added.

9. Transfer 750 µL sample to a HiBind® DNA Mini Column inserted in a 2 mL Collection Tube.

10. Centrifuge at 10,000 x *g* for 1 minute at room temperature.

11. Discard the filtrate and reuse the Collection Tube.

12. Repeat Steps 9-11 until all of the remaining sample has been transferred to the HiBind® DNA Mini Column.

13. Add 750 µL DNA Wash Buffer.

    Note: DNA Wash Buffer must be diluted with 100% ethanol before use. Please see Page 4 for instructions.

14. Centrifuge at 10,000 x *g* for 1 minute at room temperature.

15. Discard the filtrate and reuse the Collection Tube.

16. Centrifuge at 10,000 x *g* for 2 minutes to dry the column.

    Note: This step is critical for removal of trace ethanol that may interfere with downstream applications**.**

17. Transfer the HiBind® DNA Mini Column to a sterile 1.5 mL microcentrifuge tube.

18. Add 50 µL sterile deionized H_2O (or low salt buffer) directly onto HiBind® matrix.

19. Centrifuge at 10,000 x *g* for 1 minute to elute bound DNA.

    Note: For DNA <100 bp, approximately 80% will be recovered with a single elution. Subsequent elution steps each yield 80% of the remaining bound material. For expected yields greater than 20 µg, repeat the elution with another 50 µL sterile deionized H_2O

DNA Quantification

Oligonucleotides are often quantitated in A_{260} units. Although controversial, the practical definition is that 1 A_{260} is the amount of oligonucleotide, which dissolved in 1.0 mL buffer, measured in a 1.0 cm path length cuvette, at 260 nm gives an absorbance of 1.0.

1. Dilute an appropriate amount of purified DNA in dH_20 or TE buffer. The sample should give an absorbance value between 0.1 and 0.5. However if expected yields ≤ 5 µg then observed OD values will be lower.

2. Measure the absorbance in a clean 1-cm quartz cell at 260 nm.

3. Calculate the extinction coefficient (ε) of the product by the addition of the individual extinction coefficients of every nucleotide not taking into account hypochromicity effects. The epsilon-values are listed in the table below.

Nucleoside	ε
dT	8.8 cm^2/µmol
dC	7.3 cm^2/µmol
dG	11.7 cm^2/µmol
dA	15.4 cm^2/µmol

4. Calculate the sample concentration with Lambert-Beer law:

A= [ε] oligo x C x l
A = absorbance at 260 nm
[ε] oligo = the sum of the individual extinction coefficients for each nucleotide
C = µmolar concentration
l = the path of the quartz cell (1 cm).

Note: Remember that a solution of 1 µM has 1 pmol/µL solute. For dsDNA, [ε] oligo should be doubled.

Troubleshooting

Please use this guide to troubleshoot any problems that may arise. For further assistance, please contact the technical support staff, toll free, at **1-800-832-8896**.

Problem	Cause	Solution
Little or no DNA recovered	Not enough HBC Buffer added to eluate	Measure volume of eluate and add 4.5 to 5 volumes of HBC Buffer. For DNA <100 bp it may be necessary to add more.
	DNA band not excised from gel	Inspect gel to ensure band is removed.
	Incomplete elution from acrylamide gel	Increase incubation time with Elution Buffer.
	Ethanol not added to DNA Wash Buffer	Add 100% ethanol to DNA Wash Buffer prior to use. See Page 4 for details.
	Isopropanol not added to HBC Buffer	Add isopropanol to HBC Buffer prior to use. See Page 4 for details.
	Not enough starting material	Increase starting material or assess yield by performance in downstream application.
Problem		
Poly-Gel filter unit clogged	Acrylamide gel not crushed before adding Elution Buffer	Completely mince gel fragment as indicated using razor blade or microscope slide method.